Supplementary Information
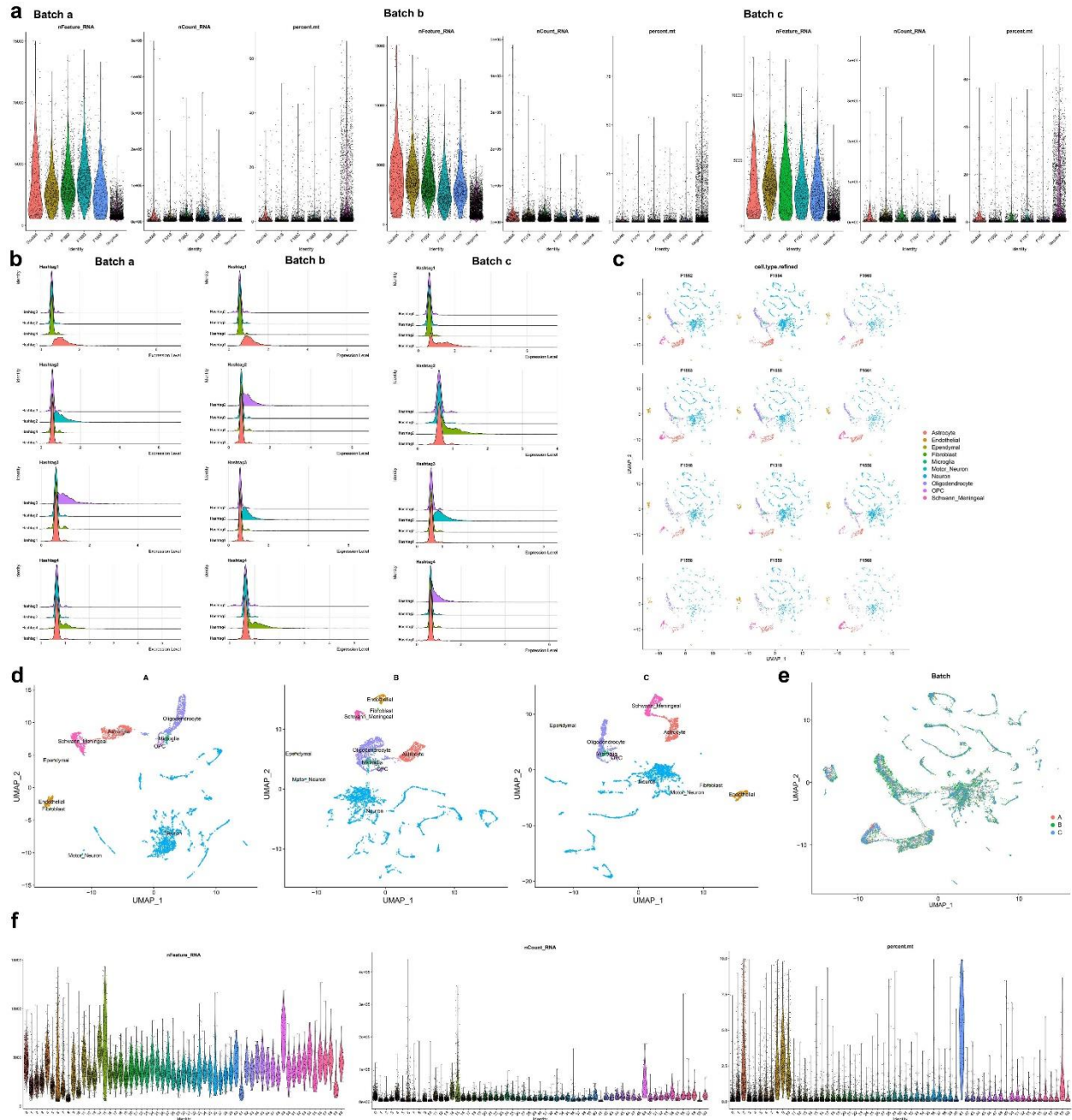
Neuronal–Glial Communication Perturbations in Murine *SOD1G93A* Spinal Cord

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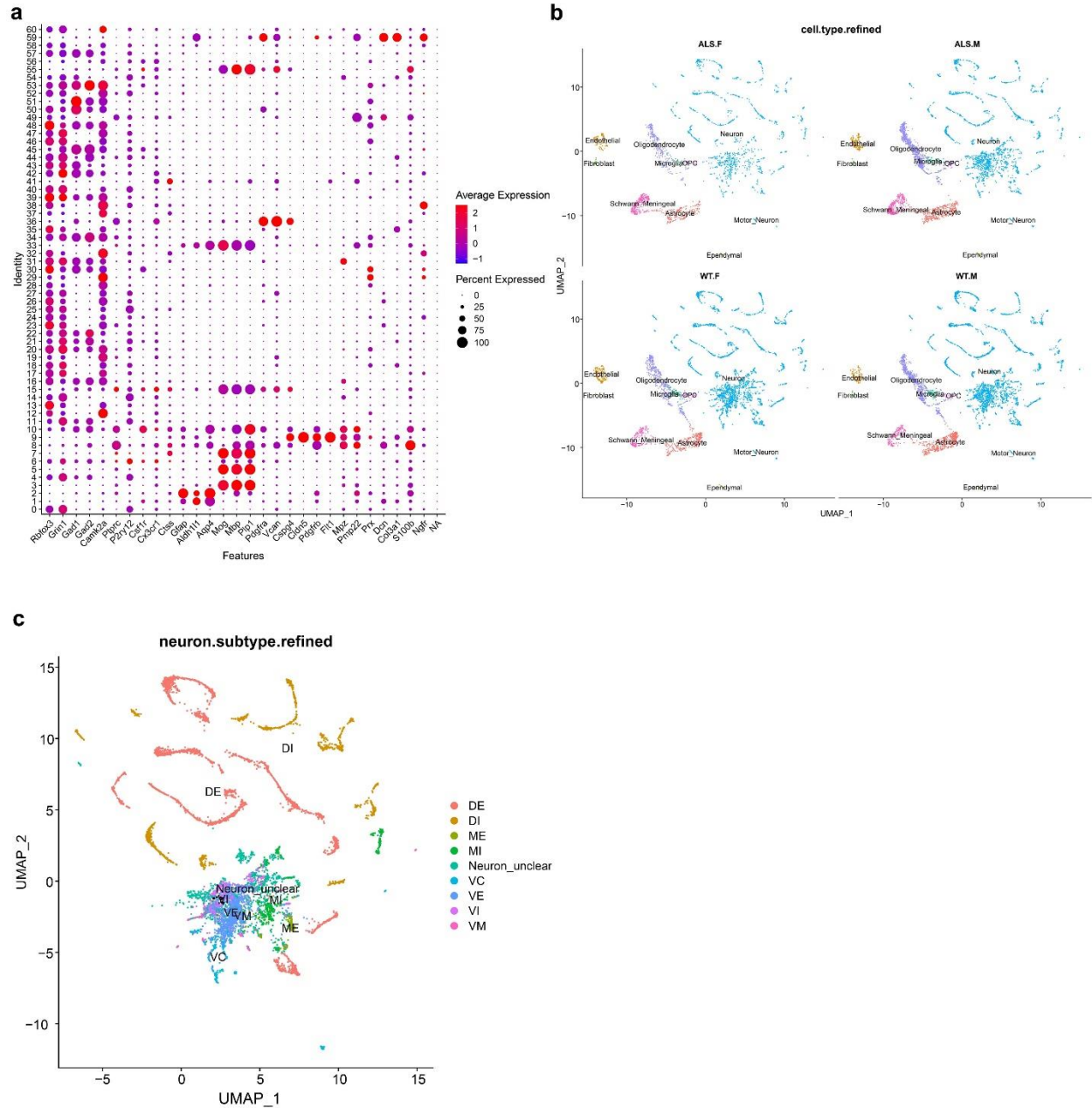
Supplementary Figure 1. Data integration of three hashtagged batches of nuclei
a. Violin plots illustrating number of total gene ("features"), total RNA counts, and percent mitochondrial data per sample or in nuclei confidently attributed to mouse samples, hashtag negative or doublet nuclei for each batch. **b.** Nuclear hashtag expression ridge plot for labeled nuclei split by unique hashtag and batch. *x*-axis: hashtag normalized hashtag expression level, *y*-axis: number of nuclei. **c.** UMAP for each sample, representing all batches (columns) and hashtags (rows). In this study each hashtag corresponds to a particular sex-genotype combination: Hashtag

1=Male WT, 2=Male *SOD1*^{G93A}, 3=Female WT and 4=Female *SOD1*^{G93A}. **d.** UMAPs for each batch after data filtering prior to data integration. **e.** UMAP of fully integrated data with nuclei colored by batch. **f.** Violin plots illustrating number of total genes ("features"), total RNA counts, and percent mitochondrial data per cluster or in nuclei confidently attributed to mouse samples, hashtag-negative or doublet nuclei. $N = 3$ independent mice per genotype per sex.

Supplementary Figure 2. Cell-type identification within integrated dataset
a. Dot plot of marker gene expression in each Seurat cluster. Clusters were labeled with cell types using a combination of marker genes and SingleR annotations. **b.** UMAP plot of cell-type labeled lumbar spinal cord nuclei split by experimental group. **c.** UMAP of neuronal sub-clusters, not including motor neurons, labeled by mapping to reference data from Sathyamurthy et al[1]. DE: Dorsal Excitatory. DI: Dorsal Inhibitory. ME: Mid Excitatory. MI: Mid Inhibitory. VE: Ventral Excitatory. VI: Ventral Inhibitory. VC: Ventral Cholinergic (non motor neurons). VM: Ventral Mixed. Unclear neurons are those not assignable to any of the above sub-types. $N = 3$ independent mice per genotype per sex.

a

b

GLUTAMATERGIC SYNAPSE
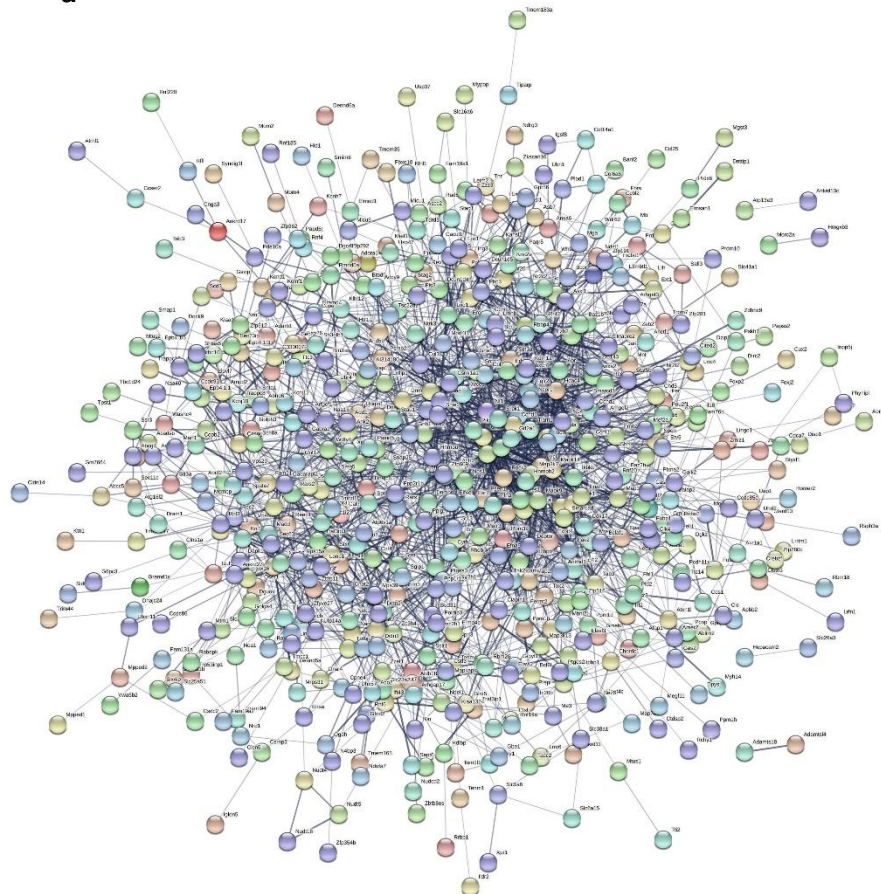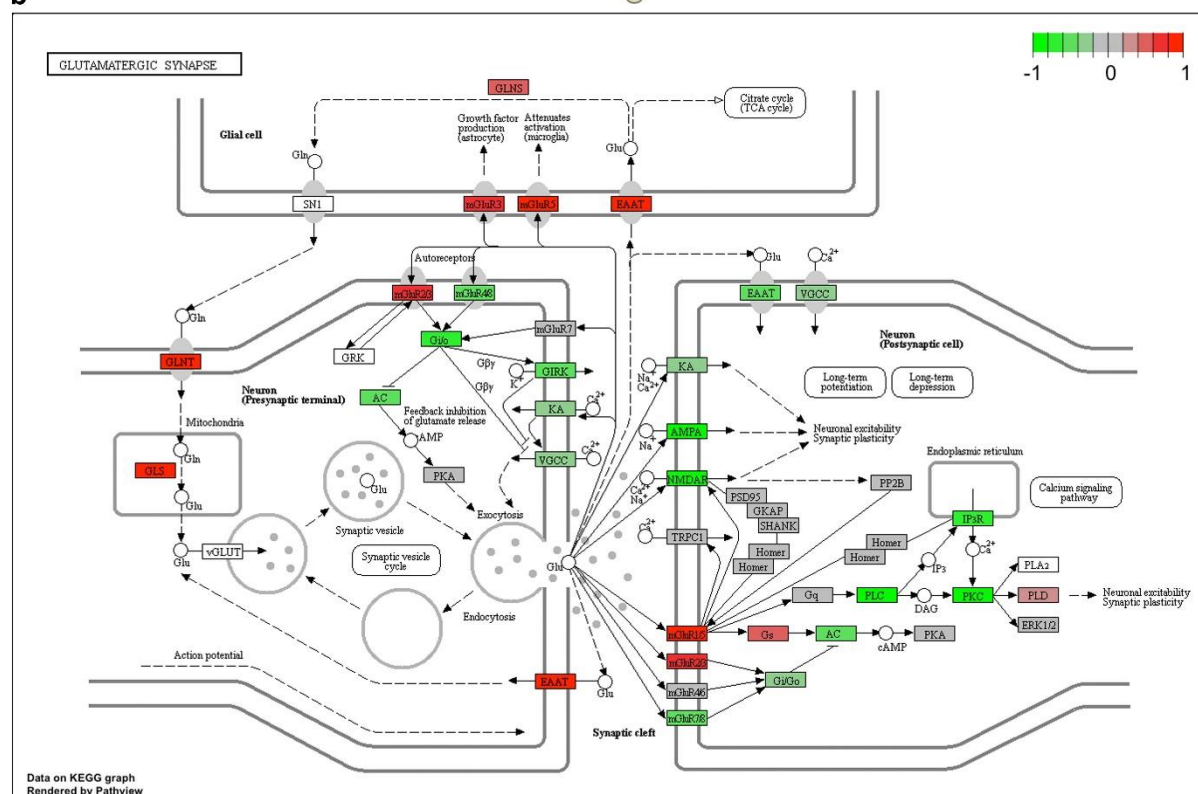
Supplementary Figure 3. SOD1^{G93A} motor neurons display altered RCOR1 regulon activity alongside perturbed glutamatergic synapse associated gene expression.
a. STRING network of protein–protein interactions for genes associated with the RCOR1 regulon. Unconnected genes are not shown. **b.** Glutamatergic synapse KEGG pathway (mmu04724) colored by log$_2$ fold change between *SOD1^{G93A}* vs. WT motor neurons. $N = 3$ independent mice per genotype per sex.

a

b

WT

SOD1^{G93A}

DAPI GFAP 4-HNE

c 4-HNE Area

d 4-HNE Intensity

e 4-HNE GFAP
Overlap Area

f 4-HNE Intensity
in GFAP Area
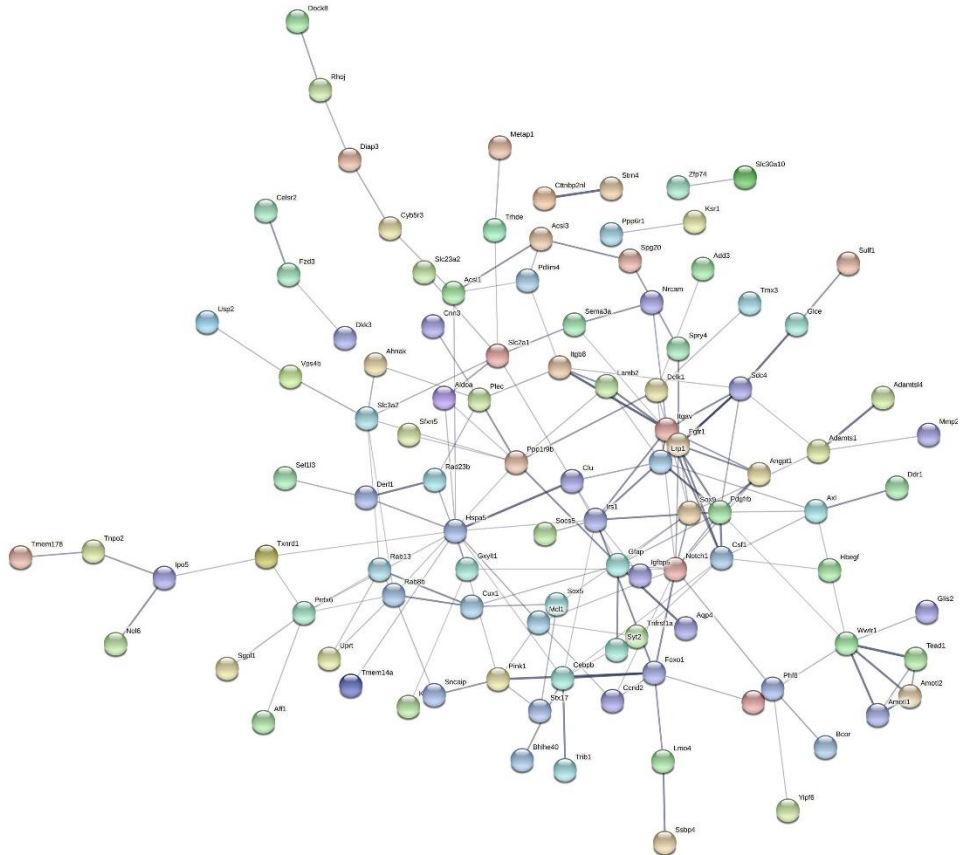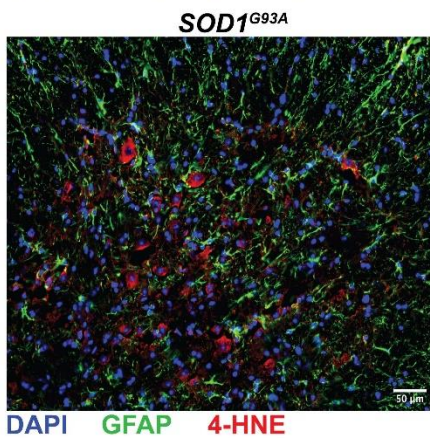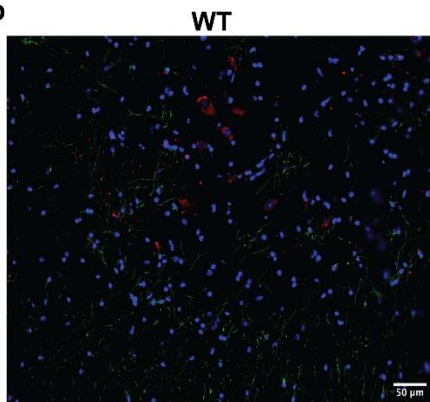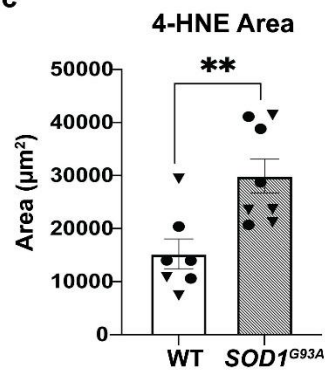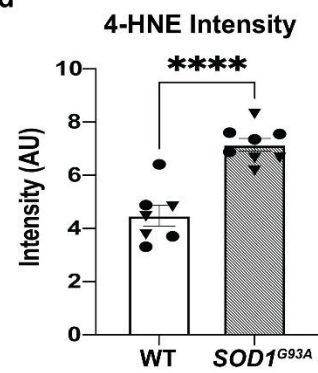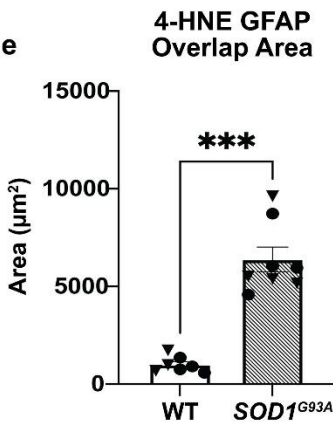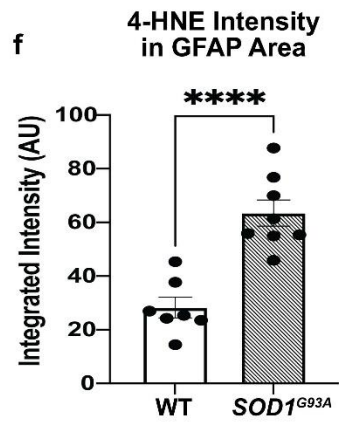
Supplementary Figure 4. SOD1^{G93A} astrocytes display increased oxidative stress by age 120 days
a. STRING network of protein–protein interactions for genes associated with the FOS regulon. Unconnected genes are not shown. **b.** Representative images of DAPI, GFAP and 4-HNE in male murine *SOD1^{G93A}* and WT lumbar spinal cord at 120 days of age. **In c-f:** Quantification of **(c)** 4-HNE area; **(d)** 4-HNE intensity; **(e)** 4-HNE and GFAP overlap area; and **(f)** 4-HNE intensity within GFAP area. In **d,f**, Independent two sample two-sided *t*-test. In **c,e**: Mann-Whitney *U*-test. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Data are presented as mean ± SEM. Specific *p* values are as follows: In c, **$p=0.0059$; in d, ****$p<0.0001$; in e, ***$p=0.0003$; and in f, ****$p<0.0001$. In **a,** $N = 3$ independent mice per sex per genotype. In **b-f**, *SOD1^{G93A}* $N = 4$ independent mice per sex, WT $N=3$ independent male and $N=4$ female mice. Male mice are represented by triangles; female mice are represented by circles.
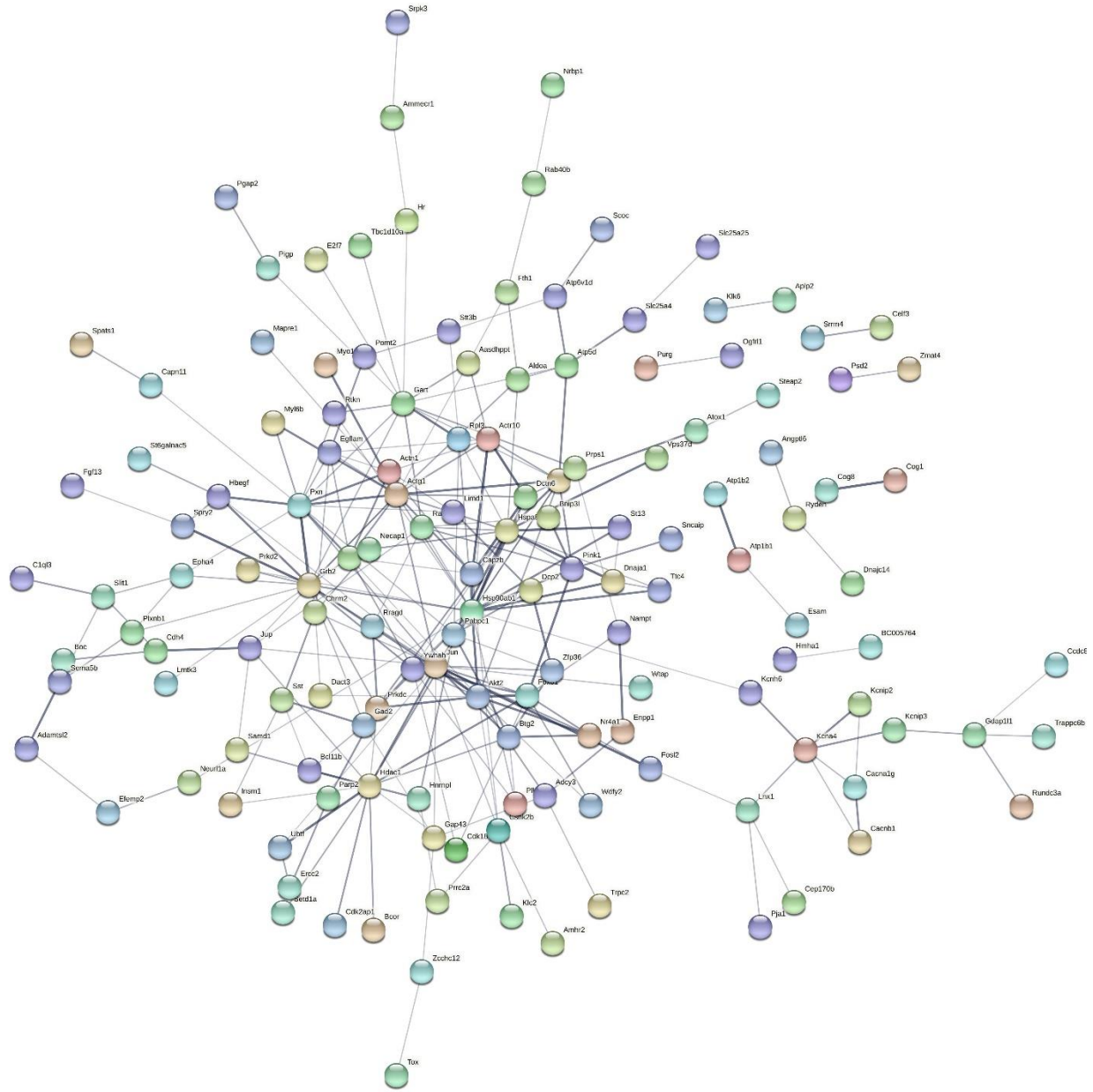
Supplementary Figure 5. Sub-cluster 0 SOD1^{G93A} microglia display up-regulated EGR1-dependent regulon activity.
STRING network of protein–protein interactions for genes associated with the EGR1 regulon. Unconnected genes are not shown. $N = 3$ independent mice per genotype per sex.
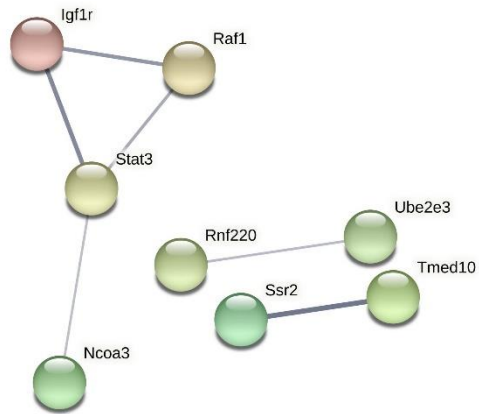
Supplementary Figure 6. Sub-cluster 0 SOD1^{G93A} oligodendrocytes display increased STAT3-dependent regulon activity.
STRING network of protein–protein interactions for genes associated with the STAT3 regulon. Unconnected genes are not shown. $N = 3$ independent mice per genotype per sex.

a   **P30**

	Astrocyte	Endothelial	Ependymal	Fibroblast	Microglia	Motor_Neuron	Neuron	Oligodendrocyte	OPC	Schwann_Meningeal
Vent_Med_White	2	0	0	0	0	0	0	0	0	0
Vent_Horn	1	0	0	0	0	0	0	0	0	0
Vent_Lat_White		1	0	0	1	2	1	1	0	1
Med_Grey	1	0	0	0	0	0	0	0	0	0
Dors_Horn	0	0	0	0	0	1	0	0	0	0
Dors_Edge		1	0	0	1	1	1	1	0	1
Med_Lat_White		1	0	0	1	2	1	1	0	1
Vent_Edge	2	1	0	0	1	1	1	1	0	1
Dors_Med_White	1	0	0	0	1	2	0	0	0	0
Cent_Can	1	0	0	0	0	0	0	0	0	0
Lat_Edge	2	1	0	0	1	1	1	1	0	1

b   **P100**

	Astrocyte	Endothelial	Ependymal	Fibroblast	Microglia	Motor_Neuron	Neuron	Oligodendrocyte	OPC	Schwann_Meningeal
Vent_Med_White	14	0	0	0	0	5	0	2	0	0
Vent_Horn		0	0	0	1	7	1	1	0	0
Vent_Lat_White	19	0	0	0	0	7	0	0	0	1
Med_Grey	25	0	0	0	1	2	2	3	0	0
Dors_Horn	12	0	0	0	1	0	1	2	0	0
Dors_Edge	11	0	0	0	1	5	1	1	0	0
Med_Lat_White	25	0	0	0	1	7	1	2	0	0
Vent_Edge	16	0	0	0	2	6	1	2	0	0
Dors_Med_White	16	0	0	0	1	3	1	1	0	0
Cent_Can	13	0	0	0	1	2	1	1	0	0
Lat_Edge	9	0	0	0	0	5	0	0	0	0

c   **P120**

	Astrocyte	Endothelial	Ependymal	Fibroblast	Microglia	Motor_Neuron	Neuron	Oligodendrocyte	OPC	Schwann_Meningeal
Vent_Med_White	29	0	0	0	1	7	1	2	0	0
Vent_Horn		0	0	0	1	22	1	2	0	0
Vent_Lat_White	28	0	0	0	1	8	1	2	0	0
Med_Grey	37	0	0	0	1	8	1	2	0	0
Dors_Horn	25	0	0	0	1	2	1	2	0	0
Dors_Edge	21	0	0	0	0	11	0	2	0	0
Med_Lat_White	29	0	0	0	1	7	1	2	0	0
Vent_Edge	22	0	0	0	1	6	1	2	0	0
Dors_Med_White		0	0	0	1	7	1	3	0	0
Cent_Can	15	0	0	0	0	3	0	0	0	0
Lat_Edge	14	0	0	0	0	9	0	1	0	0

Supplementary Figure 7. Comparison of cell type-specific alterations in SOD1^{G93A} mice vs. previously reported spatial transcriptomic changes at multiple time points.
Number of DEGs overlapping between cell type-specific snRNA analyses and anatomical regions from spatial transcriptomics data from Maniatis *et al.*[2] at **a.** P30, **b.** P100, and **c.** P120. DEGs defined by FDR < 0.1 in cell type-specific analyses and Bayes factor > 3 in anatomical locations[2].

Supplementary Dataset:

Results of Limma, ROAST, CAMERA and SCENIC analyses. N = 3 independent mice per genotype per sex. N = 76 ALS patients and N = 11 non-neurological control patients.

Supplementary References:

1   Sathyamurthy, A. *et al.* Massively Parallel Single Nucleus Transcriptional Profiling Defines Spinal Cord Neurons and Their Activity during Behavior. *Cell Rep* **22**, 2216-2225, doi:10.1016/j.celrep.2018.02.003 (2018).
2   Maniatis, S. *et al.* Spatiotemporal dynamics of molecular pathology in amyotrophic lateral sclerosis. *Science* **364**, 89-93, doi:10.1126/science.aav9776 (2019).